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SPONTANEOUS HISTAMINE SECRETION FROM MAST CELLS IN THE PRESENCE OF STRONTIUM

BY J. C. FOREMAN

From the Department of Pharmacology, University College London, London, WC1E 6BT

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SUMMARY

1. Histamine secretion from rat peritoneal mast cells occurs spontaneously in the absence of an external stimulus. Spontaneous secretion increases as the concentration of Sr in the extracellular medium is raised from 1 to 10 m-mole/l. Ca 0·1–10 m-mole/l. does not increase spontaneous secretion.

2. Spontaneous histamine secretion in the presence of Sr occurs slowly compared with evoked histamine secretion, reaching a maximum only after more than 120 min incubation with Sr 10 m-mole/l. at 37° C and pH 7·6. Phosphatidyl serine, 10 μg/ml., increases the rate of spontaneous secretion in the presence of Sr.

3. The spontaneous secretion occurring in the presence of Sr is highly dependent on the extracellular H ion concentration. Maximal secretion occurs at pH 8·4 and only a very limited secretion is detected at pH below 7·6. The rate of spontaneous secretion is also greater at higher pH. Inhibition of secretion caused by lowering the pH can be reversed by raising the Sr ion concentration over a limited range.

4. Intact glycolytic and oxidative metabolism is required for the spontaneous secretion of histamine in the presence of Sr. Removal of extracellular glucose inhibits the secretion by about 80%, and the further addition of inhibitors of oxidative phosphorylation almost abolishes the secretion.

5. Ca, Mg and Mn all inhibit the spontaneous secretion of histamine which occurs in the presence of Sr. The antagonism of the effect of Sr by Mg appears not to be competitive.

6. Dibutyryl cyclic AMP, 10 μmole/l. to 3 m-mole/l. and theophylline, 30 μmole/l. inhibit spontaneous secretion in the presence of Sr. Cyclic AMP, AMP, and cyclic GMP 10 m-mole/l. are without effect on the spontaneous secretion. The inhibitory effect of dibutyryl cyclic AMP and of theophylline are dependent on pH: greater inhibition being achieved at lower pH.

INTRODUCTION

Mast cells contain membrane bound granules in which histamine is stored. An antigen-antibody reaction on the cytoplasmic membrane leads to a release of the histamine-containing granules without a concomitant loss of cytoplasmic contents (Johnson & Moran, 1969). Release of histamine from mast cells stimulated in this way appears to show many similarities with other secretory systems (Douglas, 1968).

Histamine secretion induced by the antigen-antibody reaction requires the presence of extracellular Ca (Mongar & Schild, 1958; Lichtenstein & Osler, 1964; Greaves & Mongar, 1968; Foreman & Mongar, 1972; Yamamoto & Greaves, 1973). Sr and Ba are capable of substituting for Ca in the activation of histamine secretion (Mongar, 1970; Foreman & Mongar, 1972). Histamine secretion occurs when the Ca ionophore, A 23187, enters the mast cell membrane and transports Ca into the cell (Foreman, Mongar & Gomperts, 1973; Cochrane & Douglas, 1974). The antigen-antibody reaction increases mast cell membrane permeability to Ca and Sr (Foreman *et al.* 1973; Foreman, 1973*a*; Foreman, Hallett & Mongar, 1977*a*,*b*). The evidence supports, therefore, the hypothesis that the antigen-antibody reaction initiates histamine secretion by raising the mast cell membrane permeability to Ca or Sr, allowing passage of these ions into the cell.

In addition to the secretion evoked by the antigen-antibody reaction, a small amount of spontaneous secretion occurs in the absence of a stimulus. Spontaneous histamine secretion from mast cells occurs independently of extracellular Ca (Foreman & Mongar, 1973*a*; Foreman, 1973*b*), but the presence of extracellular Sr leads to an increase in spontaneous secretion, some of the characteristics of which are described in this paper.

METHODS

Male or female rats weighing between 150 and 350 g were obtained from a closed, random-bred colony of the Lister Hooded strain. Rats were anaesthetized in an atmosphere of nitrous oxide and air and then killed by decapitation. Four ml. saline (NaCl, 154 m-mole/l.) containing heparin 25 u./ml. was injected into the peritoneal cavity of each rat. The abdomen was massaged for about 1 min and then the fluid contained in the peritoneal cavity was withdrawn through a mid-line incision. Peritoneal washings from several rats were pooled to provide sufficient cells for an experiment. The washings contained about 5% mast cells and were divided into samples of equal volume, each sample containing 0·4–4 μg histamine. One rat provided sufficient cells for six to eight samples. The samples were centrifuged at 50 *g* for 5 min and the supernatants were discarded. The pellets were resuspended in a medium containing ions and drugs at concentrations appropriate for the experiment. The final volume of the cell suspension at this stage was usually 1 ml. The samples were placed in a water-bath at 37° C for the required incubation period and then any reaction proceeding was terminated by adding 2 ml. ice-cold HEPES-

Tyrode medium free from Ca or other alkaline earth ions. The samples were then kept on ice until they were centrifuged at 1000 *g* for 5 min. The supernatants were retained for histamine assay and the pellets were resuspended in a known volume of HEPES-Tyrode medium and heated in a water-bath at 100° C for 10 min to release residual cellular histamine.

Assay of released and residual histamine was performed biologically on the isolated guinea-pig ileum (Boura, Mongar & Schild, 1954). Sr ions and other drugs used in the experiments were not present in sufficient concentration to affect the assay of histamine. The degree of secretion is expressed as a percentage of total cellular histamine content and is calculated as the ratio:

$$\frac{\text{histamine released}}{\text{histamine released} + \text{residual cellular histamine}} \times 100.$$

The medium used for incubating the cells was based on Tyrode solution with the usual bicarbonate buffer replaced by HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acids) (Burroughs Wellcome). The medium had the following composition: NaCl 137 m-mole/l.; KCl 2·7 m-mole/l.; NaH_2PO_4 0·4 m-mole/l.; glucose 5·6 m-mole/l.; HEPES 20 m-mole/l. Alkaline earth ions were added as required. In experiments involving adjustment of pH, NaOH, 1 mole/l. or HCl, 1 mole/l. was used to bring the pH of the HEPES to the required level.

The chemicals used to prepare HEPES-Tyrode solution were of Analar quality. Theophylline and NaCN were obtained from British Drug Houses. Nucleotides, Antimycin A and 2-deoxy-D-glucose were obtained from Sigma. Phosphatidyl serine was obtained from Lipid Products as a solution in chloroform-methanol. It was prepared for experiments by evaporating the solvent in a stream of nitrogen, adding saline to produce the required concentration and mixing in a mechanical blender. Preparation of the phospholipid in this way has been found to produce an activity equivalent on a molar basis to the activity of a preparation made by sonication (Foreman, 1973*a*).

RESULTS

Concentration of Sr

The concentration range over which Sr will substitute for Ca in antigen-evoked histamine secretion is 1–10 m-mole/l. (Foreman & Mongar, 1972), and it is over this same concentration range that increase of the Sr ion concentration produces a graded increase in spontaneous histamine secretion (Fig. 1). Doubling the concentration of Sr causes an increase in secretion from 16 to 84% of maximum.

Rate of secretion

The time course of Ca ion transport into mast cells produced by the Ca ionophore, A 23187, is similar to the time course of histamine secretion induced by this ionophore: both processes being essentially complete within 5 min of the addition of the ionophore to the cells (Foreman *et al.* 1973). Similarly, antigen-evoked histamine secretion is rapid and is virtually complete within 1 min of applying the stimulus to the cells (Chakravarty, 1960; Mongar & Svec, 1972). In contrast, the spontaneous secretion of histamine in the presence of Sr ions is a slow process, being incomplete

even after 4 hr incubation at 37° C with the optimum Sr concentration of 10 m-mole/l. at pH 7·8 (Fig. 2*A*). Over the same period of time, spontaneous histamine secretion in the presence of Ca 1 m-mole/l. or in a nominally Ca-free medium increases only slightly to about 8% of total cell histamine compared with 33% on the presence of Sr (Fig. 2*A*.) In fact, the spontaneous secretion in a Ca-free medium is about twice as large as that in a medium containing Ca, 1 m-mole/l. Raising the Ca concentration to 10 m-mole/l. does not increase the spontaneous secretion of histamine.

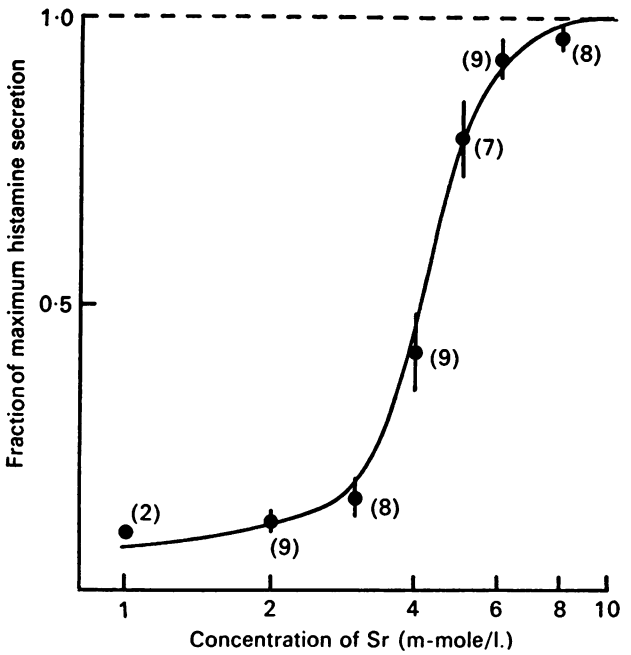

Fig. 1. Concentration–response relationship for the activation of spontaneous histamine secretion by Sr ions. Response is expressed as a fraction of the maximum histamine secretion obtainable at pH 8·4 after 120 min incubation at 37° C which had a value of 70·5 ± 3·0% (mean ± S.E.) of total cell histamine in nine experiments.

It has already been shown that phosphatidyl serine, 10 μg/ml., potentiates spontaneous histamine secretion occurring in the presence of Sr (Foreman, 1973*b*; Foreman & Mongar, 1973*a*,*b*). Fig. 2*A* shows that phosphatidyl serine increases the rate as well as the magnitude of the spontaneous secretion in the presence of Sr, maximum secretion being obtained after 90 min incubation at 37° C with an optimum concentration of Sr at pH 7·8. In contrast to its effect on antigen-evoked histamine secretion in the

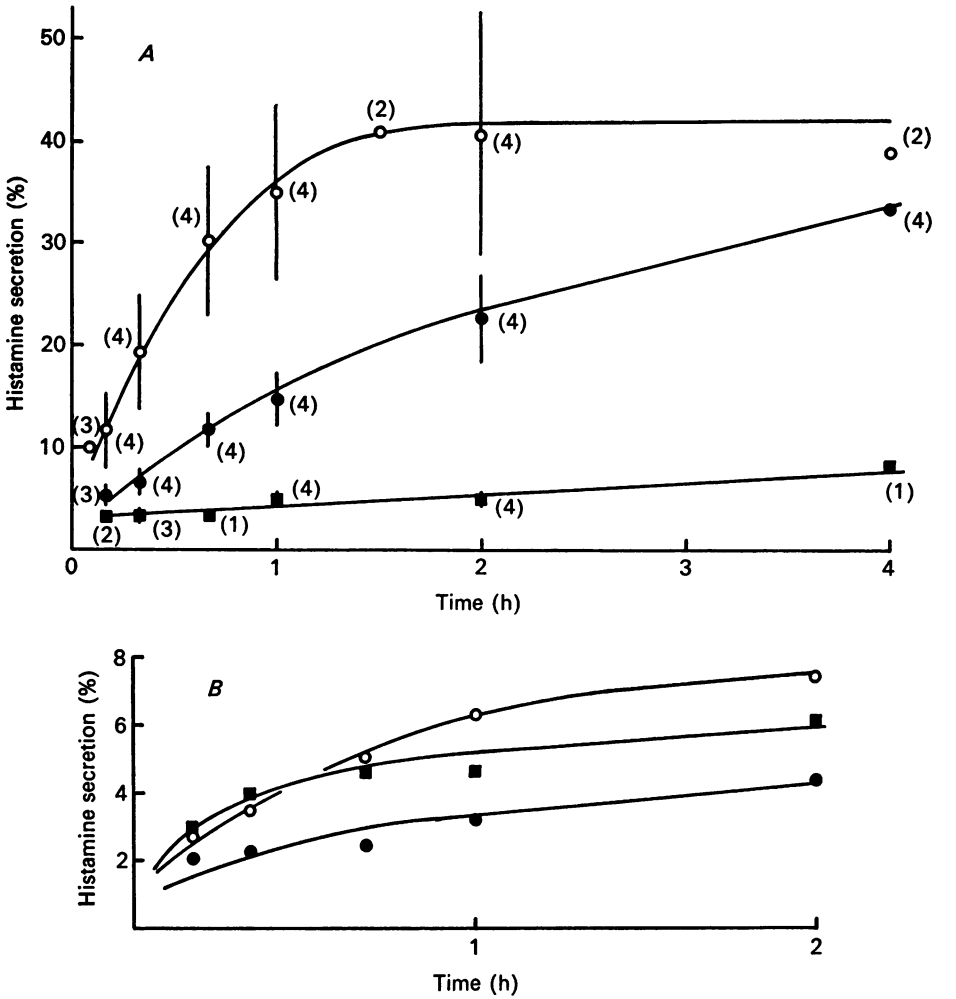

Fig. 2. *A*, time course of spontaneous histamine secretion from mast cells at 37° C, pH 7·8. ■—■, Ca, 1 m-mole/l.; ●—●, Sr, 10 m-mole/l.; ○—○, Sr, 10 m-mole/l. and phosphatidyl serine 10 μg/ml. Vertical bars represent the S.E. of mean and the numbers in parentheses indicate the number of experiments contributing to the point. *B*, rate of spontaneous histamine secretion in three separate experiments carried out at 37° C and pH 7·8. ●—●, Ca, 1 m-mole/l.; ○—○, Ca, 1 m-mole/l. and phosphatidyl serine, 10 μg/ml. ■—■, no alkaline earth ions added.

presence of Ca (Mongar & Svec, 1972), this phospholipid produces only a small increase in the magnitude of spontaneous secretion in the presence of Ca, 1 m-mole/l. (Fig. 2*B*).

The effect of H ion concentration

The original observation that the spontaneous secretion of histamine is increased in the presence of Sr was made with cells suspended in a medium at pH 7·8 (Foreman, 1973*b*). It is clear from the results shown in Fig. 3

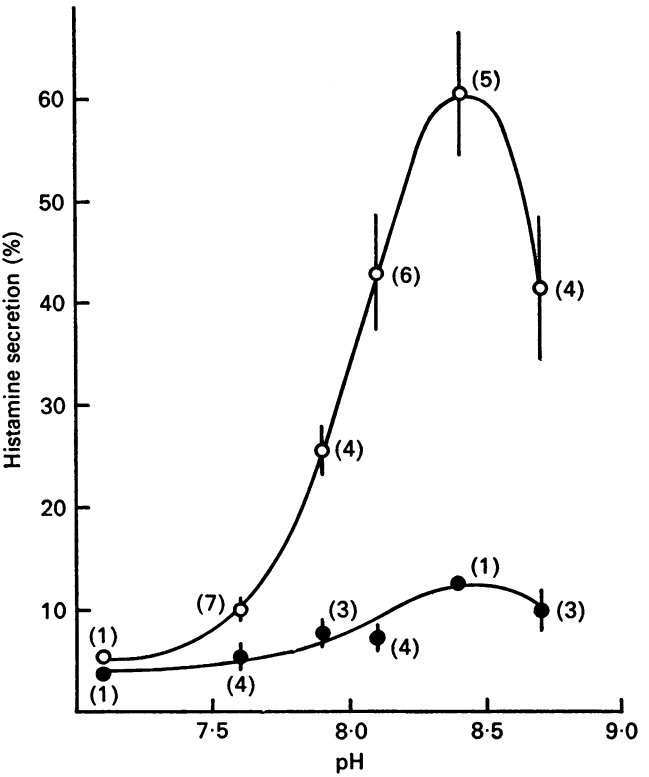

Fig. 3. Effect of H ion concentration on spontaneous histamine secretion. Cells were incubated at 37° C for 120 min. ●—●, Ca, 1 m-mole/l.; ○—○, Sr, 10 m-mole/l. Vertical bars represent S.E. of mean and the numbers in parentheses indicate the number of experiments contributing to the point.

that this was not the optimum pH for this spontaneous secretion. Raising the pH from 7·6 causes an increase in the degree of secretion up to 8·4, where an optimum secretion occurs, further increase of pH thereafter produces a fall in the secretion. A small and relatively trivial increase in

spontaneous secretion is obtained when the pH is changed over the range 7·6–8·4 in the presence of Ca, 1 m-mole/l. in place of Sr (Fig. 3).

It has been suggested that H^+ and Ca^{2+} may compete for a common site in the activation of acetylcholine release from the neuromuscular junction of the frog (Landau & Nachshen, 1975). It is also known that H^+ and Ca^{2+} interact in the antigen-evoked secretion of histamine (Mongar & Schild,

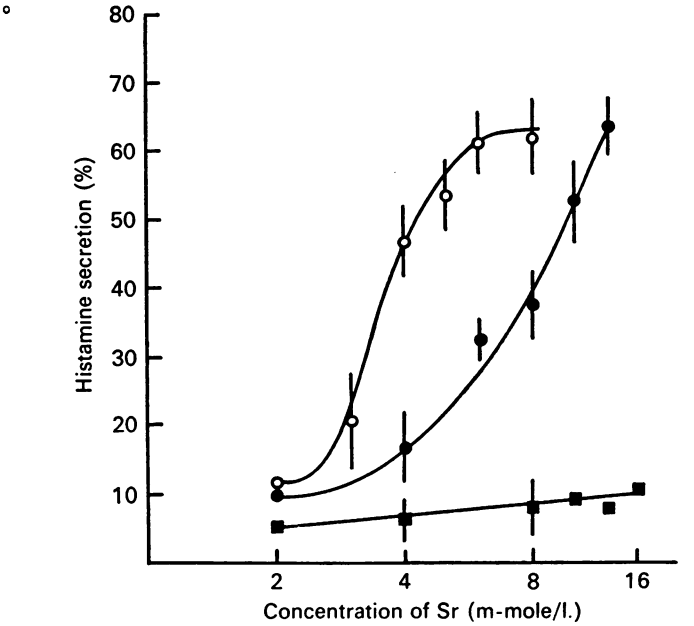

Fig. 4. Effect of H ion concentration on the concentration–response relationship between Sr and spontaneous histamine secretion. The circles are points from the same three experiments: open symbols pH 8·4 and filled symbols pH 8·1. The squares are points from four different experiments in which the pH was 7·6. Vertical bars represent S.E. of mean.

1958): as H ion concentration is increased Ca becomes less effective. It was of interest, therefore, to see if the reduction of secretion caused by lowering the pH could be counteracted by raising the Sr ion concentration. Fig. 4 shows that the inhibition of spontaneous secretion caused by lowering the pH from 8·4 to 8·1 could be reversed by raising the concentration of Sr. Change of pH by 0·3 units required a change of Sr concentration of about twofold to maintain the same secretory response. Fig. 4 shows that lowering the pH to 7·6 produced an inhibition of spontaneous secretion which showed no significant reversal even when the Sr concentration was as high as 16 m-mole/l. Complete investigation of the interaction between pH and spontaneous secretion would require the use of higher

concentrations of Sr necessitating the removal of Na to maintain isotonicity, but this may have complex effects on the secretory mechanism (Cochrane & Douglas, 1976).

In addition to increasing the magnitude of secretion, lowering the H ion concentration increases the rate of secretion (Table 1). In three experiments carried out at pH 8·4, spontaneous secretion was complete after 80 min incubation whereas at pH 7·8, the secretion is not complete after 4 hr incubation (Fig. 2*A*).

TABLE 1. Time course of spontaneous histamine secretion activated by Sr ions, 10 m-mole/l. at pH 8·4

	Time (min)							
Experiment	5	10	20	40	60	80	100	120
1	3·5	5·3	7·2	41·4	—	68·6	—	69·2
2	9·2	8·2	10·3	29·1	—	66·8	—	69·3
3	—	6·3	9·4	22·0	39·4	51·2	50·5	—

Each value for histamine release within one experiment is the mean of duplicate determinations.

TABLE 2. Effect of glucose-lack and metabolic inhibitors on spontaneous secretion activated by Sr, 10 mmole/l. with and without phosphatidyl serine (PS). Cells were incubated at 37° C for 60 min at pH 7·8

	Inhibitor	No PS	PS (10 μg/ml.)
Control histamine release	—	24 ± 2·4 %	31 ± 5·6 %
Inhibition (% of control histamine release)	Glucose-lack	81 ± 6·9	77 ± 4·2
	1 mM deoxyglucose	100 ± 4·0	100 ± 2·0
	25 μM cyanide	102 ± 4·6	100 ± 3·3
	50 μM cyanide	103 ± 4·0	102 ± 2·9

Inhibition of glycolytic and oxidative metabolism

Histamine secretion evoked by an antigen-antibody reaction on the mast cell membrane is dependent on intact glycolytic or oxidative metabolic processes within the cell. Inhibition of either of these systems alone will not inhibit completely the secretion but inhibition of both processes leads to almost total inhibition of secretion (Mongar & Schild, 1957*a*; Chakravarty, 1960, 1968; Yamasaki & Endo, 1965; Diamant, 1962). Similarly, spontaneous histamine secretion in the presence of Sr requires intact metabolic processes within the mast cell. Removal of glucose (5·6 m-mole/l.) from the extracellular medium produced about an 80 % inhibition of spontaneous secretion in the presence of Sr, and further addition of low concentrations of CN, 25 μmole/l., deoxyglucose, 1 m-mole/l. (Table 2) or

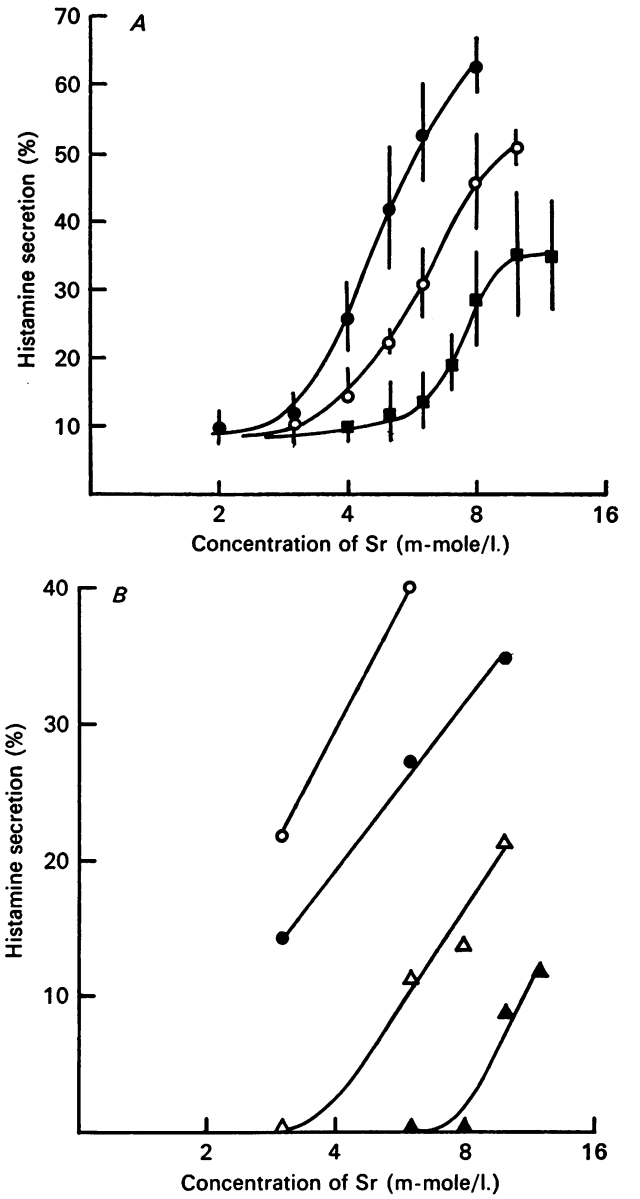

Fig. 5. *A*, effect of Mg on the concentration–response relationship for Sr and spontaneous histamine secretion. Cells were incubated for 120 min at 37° C and the pH was 8·4. ●—●, no Mg; ○—○, Mg, 6 m-mole/l.; ■—■, Mg, 12 m-mole/l. Each point is the mean from three experiments, and vertical bars represent S.E. of mean. *B*, effect of Ca on the concentration–response relationship between Sr and spontaneous histamine secretion. Cells were incubated for 120 min at 37° C and the pH was 8·1. ○—○, no Ca; ●—●, Ca; 30 μmole/l.; △—△, Ca 100 μmole/l.; ▲—▲, Ca 300 μmole/l. Results are from a single experiment and each point is the mean of duplicate

of antimycin A, 1 μmole/l. completely abolished the secretion. Even when spontaneous secretion in the presence of Sr was potentiated by the addition of phosphatidyl serine, the secretion could be completely inhibited by glucose deprivation and the addition of cyanide (Table 2). The inhibition of secretion produced by metabolic inhibitors also takes place independently of pH in the range 7·6–8·4.

The temperature dependence of antigen-evoked histamine secretion has been used as a criterion for the involvement of metabolic processes in the secretory process (Mongar & Schild, 1957*b*; Lagunoff & Wan, 1974). Spontaneous secretion in the presence of Sr is very sensitive to lowered temperature; reducing the temperature from 37° C by only 5 or 8°, resulted in 31 and 61 % inhibition of the secretion of histamine respectively.

Interaction of Sr with other divalent actions

In several secretory systems, it has been established that Mg antagonizes the action of Ca, and in some cases there is evidence to suggest that the antagonism is competitive (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Douglas & Rubin, 1963; Wernig, 1972). Experimental evidence compatible with a competitive model for the interaction between Mg and Ca and Mg and Sr has been presented for evoked histamine secretion from mast cells (Foreman & Mongar, 1972). In contrast, the antagonism by Mg of the action of Sr on spontaneous histamine secretion differs from the type of antagonism demonstrated in the case of evoked histamine secretion. Fig. 5*A* shows that the inhibition of secretion produced by Mg can only be partially reversed by increasing the Sr concentration; the maximum effect which can be achieved with Sr is reduced by the addition of Mg.

It has already been shown that Ca itself does not activate spontaneous secretion from mast cells. On the contrary, Ca inhibits the spontaneous histamine secretion which occurs in the presence of Sr (Fig. 5*B*). Again, the antagonism of the action of Sr can be reversed by raising the Sr concentration.

Mn, which inhibits the action of Ca in several secretory systems including evoked histamine secretion (Foreman & Mongar, 1973*b*; Balnave & Gage, 1973; Kanno & Nishimura, 1976; Meiru & Rahamimoff, 1972), also inhibits the spontaneous secretion of histamine in the presence of Sr. Mn, 500 μmole/l. reduced spontaneous secretion in the presence of Sr, 8 m-mole/l. from 48 to 18 %.

The action of cyclic AMP

It is well established that raised intracellular levels of cyclic AMP are associated with inhibition of antigen-evoked histamine secretion (Assem & Schild, 1971; Lichtenstein & Margolis, 1968; Koopman, Orange & Austen,

1970; Johnson, Moran & Meyer, 1974; Kaliner & Austen, 1974). Dibutyryl cyclic AMP and theophylline have been used to achieve raised intracellular levels of cyclic AMP and Fig. 6 shows that both of these agents inhibit spontaneous histamine secretion in the presence of Sr. Fig. 6*A* shows that spontaneous secretion is inhibited by dibutyryl cyclic AMP, as little as 10 μmole/l., but the concentration required to inhibit the histamine secretion depends on the pH of the incubating medium. The maximum effect of dibutyryl cyclic AMP is seen at low pH, but at this level the secretion in the absence of inhibitor is low and phosphatidyl serine is added to achieve a degree of secretion which enables the action of inhibitors to be studied. A change of pH from 7·6 to 8·1 increased the mean secretion from 12·4 $\pm$ 2·2% (phosphatidyl serine present) to 43·7 $\pm$ 6·8% while the potency of dibutyryl cyclic AMP as an inhibitor decreased by about tenfold. A further change of pH from 8·1 to 8·4 increased the secretion of histamine to 58·3 $\pm$ 6·2% and decreased the potency of dibutyryl cyclic AMP by a further tenfold.

The action of pH on the potency of dibutyryl cyclic AMP was not peculiar to that compound, and Fig. 6*B* shows that theophylline also inhibits spontaneous histamine secretion in the presence of Sr to a degree which depends on pH. Changing pH from 7·6 to 8·1 increased the histamine secretion from 10·7 $\pm$ 3·2% (phosphatidyl serine present) to 39·7 $\pm$ 7·9% while the potency of theophylline as an inhibitor of histamine secretion decreased by almost one hundredfold.

Cyclic AMP itself, AMP, cyclic GMP and dibutyryl cyclic GMP possess no capacity to inhibit or enhance spontaneous histamine secretion in the presence of Sr when they are added at concentrations up to 10 m-mole/l.

DISCUSSION

Histamine secretion evoked by an antigen-antibody reaction on the mast cell membrane is initiated by an increase in membrane permeability to Ca, and the subsequent entry of Ca into the cell from the extracellular environment (Foreman & Mongar, 1973*a*; Foreman, Mongar & Gomperts, 1973; Dahlqvist, 1974; Foreman *et al.* 1977*a*). Sr will substitute for Ca in this role (Foreman & Mongar, 1972). The results presented in this paper show that Sr will also activate histamine release from rat mast cells in the absence of any stimulus to the cell membrane.

The Ca ionophore, A 23187, will transport Sr (Caswell & Pressman, 1972) and will induce histamine secretion from mast cells in the presence of Sr (Foreman, Mongar & Gomperts, 1973). Entry of Sr into the mast cells, like the entry of Ca, is, therefore, thought to be a sufficient stimulus to trigger secretion. Spontaneous secretion in the presence of Sr may thus

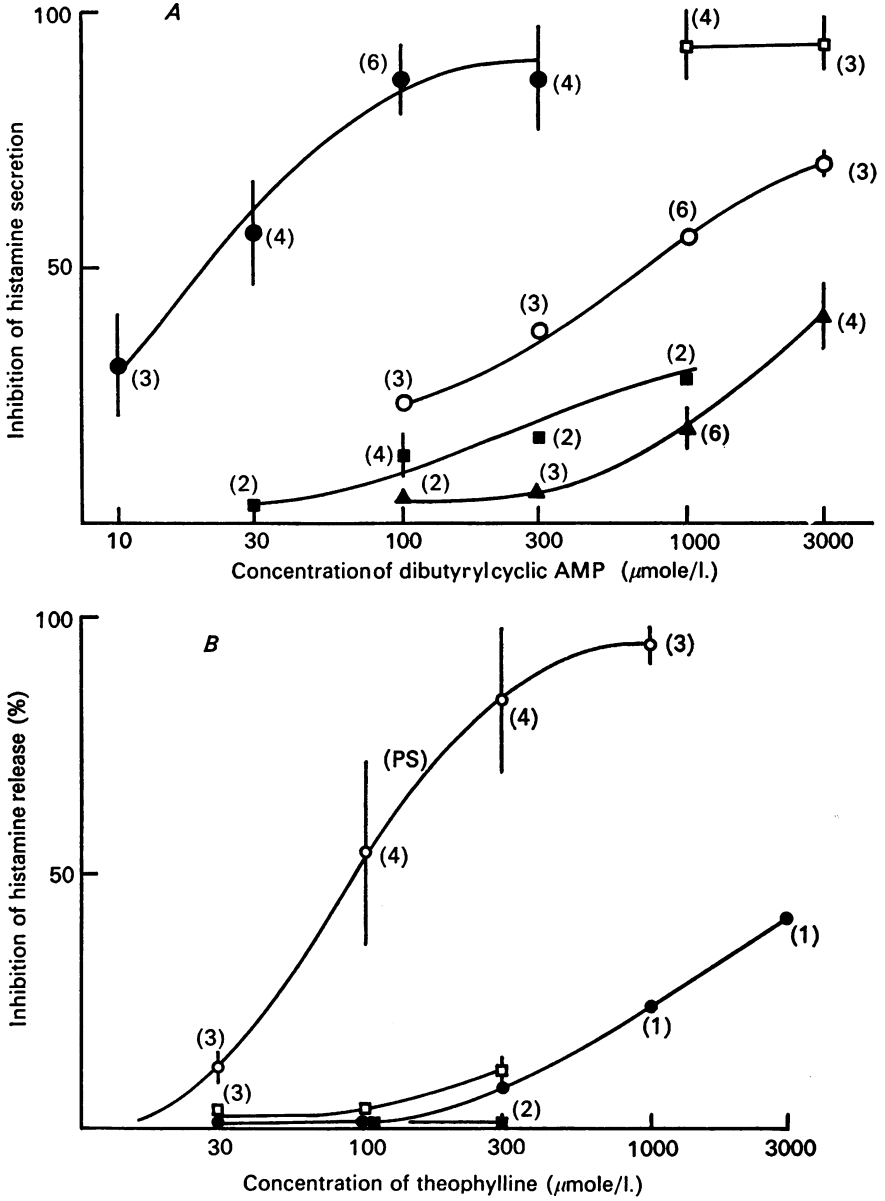

Fig. 6. For legend see opposite page.

represent leakage of the ion into the cell. The fact that Sr induces spontaneous histamine release whereas Ca does not, may reflect a greater permeability of the cell membrane to Sr which has a smaller hydrated ionic radius compared with Ca. In addition, it might reflect the inability of the normal intracellular mechanism for sequestering Ca to deal with Sr. Whereas this sequestering mechanism would normally maintain low intracellular levels of Ca, it might allow Sr levels to rise sufficiently high for secretory activity to be triggered.

The concentration of Sr which activates spontaneous secretion is the same as that which activates antigen-evoked histamine secretion (Foreman & Mongar, 1972), but the rate of spontaneous secretion in the presence of Sr is very slow compared with the rate of secretion following antigen stimulation; the latter being complete within 1 min (Mongar & Svec, 1972). Furthermore, the ionophore A 23187 transports Sr into the mast cell and releases histamine, both events having a similar time course and being complete within minutes rather than the hours over which spontaneous secretion occurs (Foreman, 1973*a*). The identical concentrations of Sr required to activate release in these different situations may indicate a common site of action, while the slow secretion occurring when Sr is present in the absence of either membrane stimulation or ionophore, could reflect a slow leakage of the ion into the cell. Evidence is presented elsewhere to show that mast cells accumulate Sr slowly in contrast to Ca which they fail to accumulate (Foreman *et al.* 1977*b*). Furthermore, no matter whether Sr enters the cell by slow accumulation in the resting cell or by fast entry into stimulated cells, the degree of secretion which follows is directly proportional to the amount of Sr accumulated by the cell (Foreman *et al.* 1977*b*).

Fig. 6. *A*, concentration–response relationship for inhibition by dibutyryl cyclic AMP of spontaneous histamine secretion activated by Sr, 10 m-mole/l. Cells were incubated at 37° C for 120 min. □—□, pH 7·6; ●—●, pH 7·6 with phosphatidyl serine, 10 μg/ml.; ○—○, pH 8·1; ▲—▲, pH 8·4; ■—■, pH 8·4 with phosphatidyl serine 10 μg/ml. Vertical bars represent S.E. of mean and the figures in parentheses are the numbers of experiments contributing to the point.

B, concentration–response relationship for inhibition by theophylline of spontaneous histamine secretion activated by Sr, 10 m-mole/l. Cells were incubated at 37° C for 120 min. ○—○, pH 7·6 with phosphatidyl serine, 10 μg/ml. ●—●, pH 8·1; ■—■, pH 8·4: □—□, pH 8·4 with phosphatidyl serine, 10 μg/ml. Vertical bars represent S.E. of mean and the figures in parentheses are the numbers of experiments contributing to the point.

The degree of inhibition is calculated by expressing the reduction of histamine secretion in the presence of inhibitor as a % histamine secretion occurring when no inhibitor was present. Values for histamine secretion in the absence of inhibitor are given in the text.

Histamine secretion evoked by antigen in the presence of Ca is dependent on intact glycolytic and oxidative metabolism for the generation of ATP (Diamant, Norn, Felding, Olsen, Ziebell & Nissen, 1974; Johansen & Chakravarty, 1972; Peterson, 1974). Similarly, spontaneous secretion in the presence of Sr is inhibited when these metabolic pathways are blocked. In addition, spontaneous secretion is sensitive to lowering of the temperature below 37° C which is similar to the behaviour of the antigen-evoked secretory response in the presence of Ca (Mongar & Schild, 1957*b*).

Activation of antigen-evoked secretion by either Ca or Sr is antagonized by Mg, and the kinetics of this antagonism are compatible with a competitive model for the interaction of Ca and Mg with a binding site (Foreman & Mongar, 1972). It has been suggested that magnesium competes with Ca for the transport site or gate through which Ca enters the cell (Rubin, Feinstein, Jaanus & Pamre, 1967). In the case of spontaneous secretion in the presence of Sr, the kinetics of the Sr–Mg interaction do not appear to fit with a simple competitive model, since the inhibitory effect of Mg cannot totally be overcome by increasing the concentration of Sr. It is, therefore, possible that the binding site involved in spontaneous secretion is different from the site involved in antigen-evoked secretion.

Interaction between Ca and Sr which are both agonists in the evoked secretion of histamine also seems to behave according to a simple competitive model (Foreman & Mongar, 1972). Although the results presented in this paper show that Ca antagonizes the action of Sr in spontaneous secretion, the concentrations of Ca which inhibit the effect of Sr in spontaneous secretion are about 1 order of magnitude less than the concentrations which interact with Sr in evoked secretion. Thus, again it is possible that the binding site involved in spontaneous secretion is different from that involved in evoked secretion.

Mn inhibits many Ca dependent processes, including antigen-evoked histamine secretion, and it probably acts by preventing Ca entry into cells. It has been shown in this paper that it inhibits spontaneous secretion in the presence of Sr.

So far as events occurring after Ca or Sr entry into the cell, such as the involvement of an intact source of ATP supply, are concerned, spontaneous and evoked secretion are similar, but there seem to be important differences between these two types of secretion when the nature of the membrane binding site or gate is considered. It has been shown that cyclic AMP inhibits antigen-evoked histamine secretion by lowering the membrane permeability of the antigen-stimulated cell to calcium: the nucleotide is thought to act on the calcium gate opened by antigen stimulation (Foreman, Garland & Mongar, 1976). If spontaneous secretion in the presence of Sr resulted from the passage of Sr ions through this gate, then cyclic AMP

might be expected to inhibit the spontaneous secretion. In fact, the results show that inhibition of spontaneous secretion by agents which raise intracellular levels of cyclic AMP is not complete at the pH at which the secretion is maximal. At the pH at which antigen-evoked secretion is maximal, spontaneous secretion in the presence of Sr is at a low level but it is more sensitive to inhibition by cyclic AMP. These results can be explained if Sr uses two routes of entry into the mast cell: (1) the antigen-operated gate which must be assumed to have resting permeability to Sr and not Ca, and (2) an alternative pathway which may be a passive conductance or perhaps more likely an ion exchange process in the mast cell membrane which depends on the extracellular pH and is selective to strontium relative to Ca. Thus, at low pH the alternative pathway is inoperative and Sr enters the cell through the gate which is normally operated by antigen: this gate is blocked by cyclic AMP. However, at high pH the alternative pathway dominates and accounts for the greater part of the spontaneous secretion which cannot be blocked by cyclic AMP because it is the other, antigen-operated channel which is sensitive to this nucleotide. The inhibition of spontaneous secretion by higher concentration of cyclic AMP seen at the greater pH is not an effect on ion transport but on some other stage in the secretory process (Foreman *et al.* 1977*b*). The non-competitive interaction between Sr and Mg in spontaneous secretion in contrast to the competitive interaction in evoked secretion also might be explained in terms of the different characteristic of the alternative pathway relative to the antigen-operated gate. Similarly, the low concentrations of Ca needed to block this conductance contrast with the concentrations of Ca which interact with Sr in evoked secretion. It must be pointed out that there is an alternative explanation for the variation in the inhibition caused by dibutyryl cyclic AMP at different pH. Change in pH necessarily changes the degree of secretion and thus it is possible that the level of inhibition by dibutyryl cyclic AMP is a function of the degree of secretion rather than the mechanism involved at different pH.

Phosphatidyl serine enhances antigen-evoked histamine secretion in the presence of Ca (Goth, Adams & Knoohuizen, 1971; Mongar & Svec, 1972; Foreman & Mongar, 1973*a*). The phospholipid increases the rate and magnitude of spontaneous secretion in the presence of Sr, probably by increasing the membrane permeability to Sr.

In conclusion, it is proposed that spontaneous histamine secretion which occurs when Sr is present in the extracellular medium is the result of entry of the ion into the mast cell. The results are consistent with a model involving two routes for entry of Sr into the cell: a pH-dependent alternative pathway, insensitive to cyclic AMP, and an antigen-operated gate which possesses resting permeability to Sr and is blocked by cyclic AMP.

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